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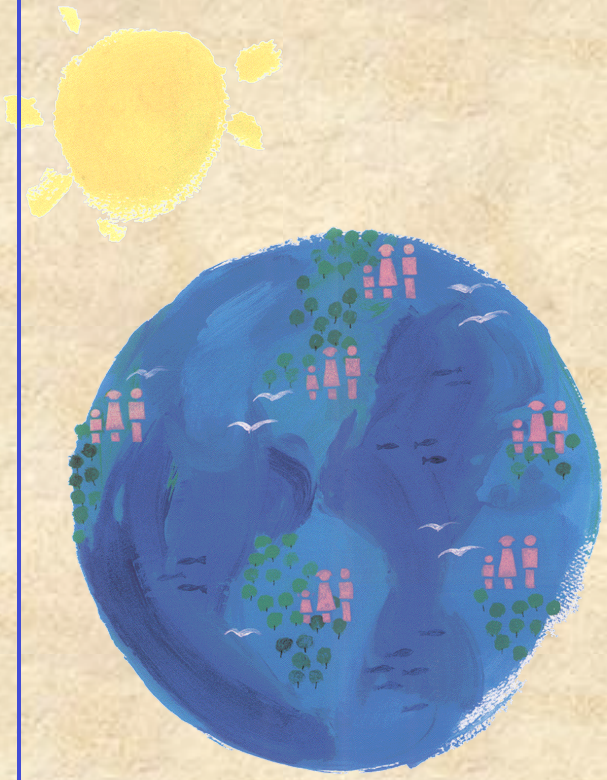

Current Ocular Toxicity Regulatory Testing Procedures

Debbie McCall, U.S. EPA

January 11, 2005

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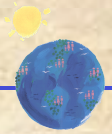
Statutes and Regulations Requiring Ocular Corrosivity / Irritation Testing

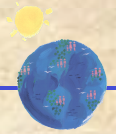
Agency	Authority	Regulation	Guideline
EPA	FIFRA (1947) TSCA (1977)	40CFR	OPPTS 870.2400
CPSC	FHSA (1964)	16CFR1500	16CFR1500.42
FDA	FDCA (1938)	21CFR	16CFR1500.42
OSHA	OHSA (1970)	??	16CFR1500.42
EU	Council Directive 67/548/EEC	Commission Directive 2004/73/EC	Annex V B.5
OECD	-	-	Test Guideline 405

CFR: Code of the Federal Register; CPSC: Consumer Product Safety Commission; EPA: Environmental Protection Agency; EU: European Union; FDA: Food and Drug Administration FDCA: Food, Drug and Cosmetic Act; FHSA: Federal Hazardous Substances Act; FIFRA: Federal Insecticide, Fungicide, and Rodenticide Act; OECD: Organisation for Economic Co-ordination and Development; OPPTS: EPA, Office of Prevention, Pesticides, and Toxic Substances; OSHA: Occupational Safety and Health Administration; TSCA: Toxic Substances Control Act



The *In Vivo* Rabbit Test

- Healthy adult albino rabbits (e.g., White New Zealand)
- 0.1 mL or 0.1 g instilled into the conjunctival sac of 1 eye
  - The untreated eye serves as a control
- Observation for at least 3 days, and may extend up to 21 days to evaluate for reversibility/irreversibility of effects
- Some regulatory authorities permit the use of a single animal to screen for corrosive effects.
  - If severe effect is seen, no further testing.
  - If no severe effects, up to 2 additional animals tested to confirm results.
  - Additional animals may be necessary to confirm weak or equivocal responses.

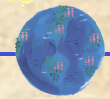


Relevant Testing Guidelines

Test Method Component	EPA*	EU*	FHSA*	OECD*
Number of Animals	n = 1 to screen for corrosive, then n ≥ 2	n = 1 to screen for corrosive, then n ≥ 2	≥ 6	n = 1 to screen for corrosive, then n ≥ 2
Quantity	0.1 mL or 0.1 g	0.1 mL or 0.1 g	0.1 mL or 0.1 g	0.1 mL or 0.1 g
Observation Times	1-72 hr (up to 21 days)	1-72 hr (up to 21 days)	1-3 days (up to 7 days)	1-72 hr (up to 21 days)
Post-dosing Irrigation	24 hr**	Liquids: 24 hr Solids: 1 hr	24 hr	Liquids: 24 hr Solids: 1 hr
Use of Anesthetics	May be used prior to dosing if pain anticipated	May be used prior to dosing if pain anticipated	May be used prior to dosing if pain anticipated	May be used prior to dosing if pain anticipated

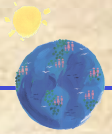
*EPA: TG OPPTS 870.2400 (1998); EU: Annex V B.05 (2004); FHSA: 16CFR1500.42 (2003); OECD: TG 405 (2002)

**For substances shown to be irritating by this test, additional testing using animals with eyes washed 30 seconds after instillation may be indicated



Rabbit Eye Test Method Scoring (I)

- Eyes are subjectively evaluated using the Draize method for three endpoints:
  1. Corneal opacity
  2. Iris effects
  3. Conjunctival effects



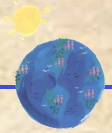
Rabbit Eye Test Method Scoring (II)

1. Cornea

- Degree of opacity
  - 1 = Scattered or diffuse area – details of iris visible
  - 2 = Easily discernible translucent areas – details of iris slightly obscured
  - 3 = Opalescent areas, no details of iris visible, size of pupil barely discernable
  - 4 = Opaque – iris not visible

- Area of cornea involved*
  - 1 = One quarter (or less) but not zero
  - 2 = Greater than one quarter, but less than one half
  - 3 = Greater than one half, but less than three quarters
  - 4 = Greater than three quarters, up to whole area

*Not used for regulatory hazard classification.



Rabbit Eye Test Method Scoring (III)

2. Iris
   - 1 = Folds above normal, congestion, swelling, circumcorneal injection (any one or all of there, or combination of any thereof), iris still reacting to light
   - 2 = No reaction to light, hemorrhage, gross destruction (any one or all of these)

Rabbit Eye Test Method Scoring (IV)

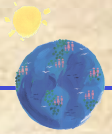
3. Conjunctiva

- Redness
  - 1 = Vessels definitely injected above normal
  - 2 = More diffuse, deeper crimson red, individual vessels not easily discernable
  - 3 = Diffuse, beefy red

- Chemosis
  - 1 = Any swelling above normal (includes nictitating membrane)
  - 2 = Obvious swelling with partial eversion of the lids
  - 3 = Swelling with lids about half closed
  - 4 = Swelling with lids half to completely closed

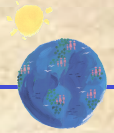
- Discharge*
  - 1 = Any amount different from normal
  - 2 = Discharge with moistening of lids and hairs adjacent to the lids
  - 3 = Discharge with moistening of lids and considerable area around the eye

*Not used for regulatory hazard classification.



Test Guidelines: Summary

- All four test guidelines are based on the original method of Draize et al. (1944)
- FSHA requires the greatest number of animals in an initial test (n = 6)
  - EPA, EU, and OECD recommend up to 3 animals in an initial test (with the possibility of only one animal classifying a corrosive substance).
- All four test guidelines permit the use of anesthetics.
  - Not recommended for routine use, only when pain is anticipated
- EPA, EU, and OECD requires studies to be carried out to 21 days to evaluate reversible/irreversible effects, while FHSA only requires observations out to 3 days.
- All four test guidelines allow irrigation of eyes after 24 hr
  - EU and OECD allows for irrigation at 1 hr for solid substances



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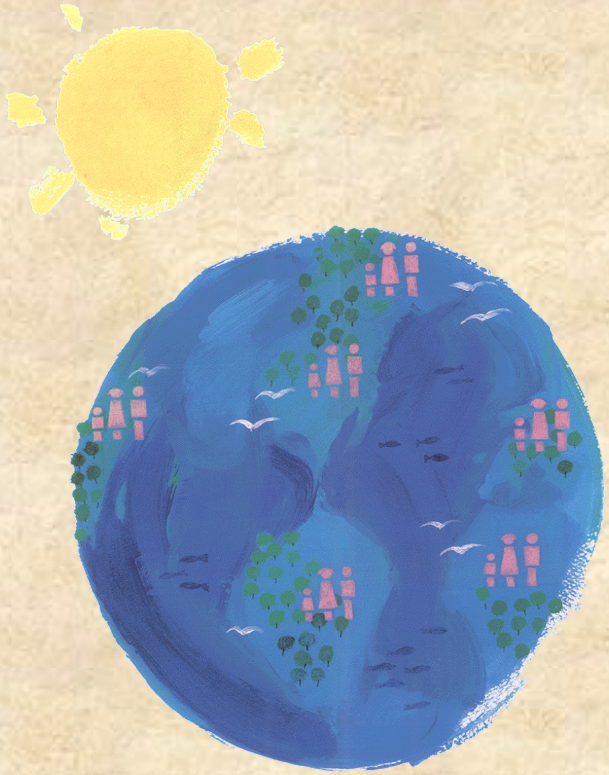

Overview of Ocular Hazard Regulatory Testing Requirements and Classification Schemes

Debbie McCall, U.S. EPA

January 11, 2005

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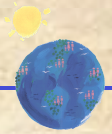
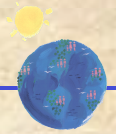
Background

- Ocular toxicity classification definitions and criteria vary among regulatory hazard classification systems (EPA, EU, GHS, FHSA)
- All current ocular toxicity classification systems are based on the Draize rabbit eye test method (Draize et al. 1944) and scoring system



Classification Systems

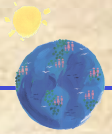


EPA Classification System (1996)

- At least 3 animals per test (one-animal screen permitted)
- Maximum score in any animal used for classification
  - Positive: CO or IR ≥ 1 or CC or CR ≥ 2
- Most severe response used for classification of substance

EPA Category	*In Vivo* Effect
I	Corrosive; (irreversible) corneal involvement or irritation persisting more than 21 days
II	Corneal involvement or irritation clearing in 8-21 days
III	Corneal involvement or irritation clearing in ≤ 7 days
IV	Minimal effects clearing within 24 hr.

CC: Conjunctival Chemosis; CO: Corneal Opacity; CR: Conjunctival Redness; IR: Iritis



EPA Labeling

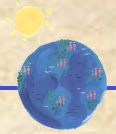
Cat.	Signal Word	Statements	Protective Equipment/Actions
I	DANGER	Corrosive. Causes irreversible eye damage. Do not get in eyes or on clothing.	Wear protective [eyewear goggles, face shield, or safety glasses]. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, or using tobacco. Remove and wash contaminated clothing prior to reuse.
II	WARNING	Causes substantial but temporary eye injury. Do not get in eyes or on clothing	Same as Category I above
III	CAUTION	Causes moderate eye irritation. Avoid contact with eyes or clothing.	Wear protective [eyewear goggles, face shield, or safety glasses]. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, or using tobacco.
IV	(CAUTION optional)	None required	None required, but may chose cat. III

EU Classification System (2001)

- At least 3 animals per test (one-animal screen for corrosive effect permitted)
- Two Possibilities for Classification
  - If > 3 animals, mean study values (each endpoint averaged over days 1-3 for all animals) used
  - If 3 animals, individual animal mean values (each endpoint averaged over days 1-3) used

EU Category	# Animals	*In Vivo* Effect
R41	> 3	Mean* study value where CO ≥ 3 or IR ≥ 1.5
R41	3	≥ 2 animals with individual means* of CO ≥ 3 or IR ≥ 2 OR ≥ 1 animal with CO or CC ≥ 2, CR ≥ 2.5, or IR ≥ 1 at end of observation (usually Day 21)
R36	> 3	Mean* study value where 2 ≤ CO < 3 or 1 ≤ IR < 1.5 or CR ≥ 2.5 or CC ≥ 2
R36	3	≥ 2animals with individual means* of 2 ≤ CO < 3 or 1 ≤ IR < 2 or CR ≥ 2.5 or CC ≥ 2

*Mean calculated over days 1-3
CC: Conjunctival Chemosis; CO: Corneal Opacity; CR: Conjunctival Redness; IR: Iritis



GHS Classification System (2003)

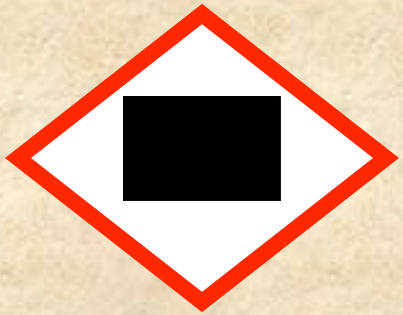
- Is test method neutral
- Classification based on severity of effect and reversibility of the effect

Category	*In Vivo* Effect
1	≥ 1 animal with CO = 4 at any time OR ≥ 2 animals with mean* CO ≥ 3 or IR ≥ 1.5 OR ≥1 animal at day 21 with CO or IR ≥ 1 or CC or CR≥ 2
2A	≥ 2 animals with mean* CO or IR ≥ 1 or CC or CR≥ 2 which reverses within 21 days.
2B	≥ 2 animals with mean* CO or IR ≥ 1 or CC or CR≥ 2 which reverses within 7 days.

*Mean values calculated over days 1-3
CC: Conjunctival Chemosis; CO: Corneal Opacity; CR: Conjunctival Redness; IR: Iritis

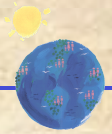
GHS Labeling

Category	Symbol	Signal Word	Label Statement
1	Corrosive symbol	Danger	Causes severe eye damage
2A	Exclamation mark	Warning	Causes severe eye irritation
2B	No symbol used	Warning	Causes eye irritation



FHSA Classification System (1995)

- At least 6 animals per test
- Corrosive: ≥ 1 animal with destruction or irreversible alterations at the site of contact
- For irritants, maximum score in any animal on any day used for classification
  - Positive: CO or IR ≥ 1 or CC or CR ≥ 2
- Testing may be carried out in multiple tiers (6 animals/tier)
  - Tier 1:
    - ≥ 4 positive animals = Irritant
    - 2-3 positive animals = Go to Tier 2
    - 1 positive animal = negative
  - Tier 2:
    - ≥ 3 positive animals = Irritant
    - 1-2 positive animals = Go to Tier 3
    - 0 = negative
  - Tier 3:
    - 1 positive animal = Irritant



Classification Systems: Summary

- EPA, EU, and GHS allow for classification of corrosive based on a one-animal screen. If the initial animal indicates corrosivity, no additional testing is required.
- Classification according to EPA and FHSA is based on the most severe lesion in any animal and on any day.
- Classification according to EU and GHS takes into account the most severe mean scores over days 1-3, in addition to persistent lesions.
- All four systems have only 1 classification for ocular corrosives/severe irritants.
  - However, different numbers of classifications for nonsevere irritants
    - EPA: n = 3 (Category II, III, or IV)
    - EU: n = 1 (R36)
    - FHSA: n = 1 (Irritant)
    - GHS: n = 2 (Category 2A or 2B)